ORIGINAL ARTICLE

Relation of hepatitis C virus genotypes to risk factors and hepatic disease in Spanish patients

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Objective: To ascertain the prevalence of hepatitis C virus (HCV) genotypes in Spain and their distribution by risk factors.

Methods: The study covered 216 patients with hepatitis C. Of these, 63 were intravenous drug users (IVDU), 44 had received transfusions, and 30 were hemodialyzed, and in 79 the risk factors were unknown. Antibodies against HCV were detected by second-generation enzyme immunoassay (EIA) and confirmed by immunoblot. HCV RNA presence was investigated by reverse transcription–polymerase chain reaction (RT-PCR), and a reverse hybridization test of the amplifications was used for the genotyping.

Results: The most frequently encountered genotypes were 1b (48.1%), 1a (21.3%) and 3a (11.1%). HCV genotypes 1a (42.8%) and 3a (20.6%) were the most prevalent genotypes in IVDU patients, while 1b was the most frequent in patients with unknown risk factors (62.0%), transfused patients (68.1%) and hemodialyzed patients (50.0%). Mixed infections were detected in nine cases (4.1%); three appeared in IVDU patients (4.7% of the total IVDUs), two in transfused patients (4.5%) and four (50%) in patients with unknown risk factors. No statistically significant differences were found in average ages of the IVDU patients with different genotypes. Non-IVDU patients having genotype 3a presented the lowest average age of all. No significant statistical differences were observed in alanine aminotransferase levels among patient groups with different genotypes ($p>0.05$ in all cases). Subtype 1b was present in six of the seven cases of cirrhosis (85.7%) and in nine of the 18 cases of active chronic hepatitis (50.0%).

Key words: Hepatitis C, HCV genotypes, epidemiology

INTRODUCTION

Since the discovery of the hepatitis C virus (HCV) in 1989 by investigators at Chiron Corporation [1], a great deal of information about the genetic diversity of HCV has appeared in world literature. The first evidence of this diversity arose from the comparison of a Japanese isolate (HCV J1) and an American one (HCV-1) [2], which revealed differences in the NS3 and NS4 regions. Later on, differences were demonstrated in the NS5 [3] and envelope [4] regions, suggesting the existence of multiple HCV types.

The immediate consequence of discovering the existence of HCV genotypes was the investigation of their relation to varied aspects of the infection. One of the aspects most studied has been the geographic distribution of the different genotypes, which have been found to be widely spread. This is especially true of genotype 1b, which seems to have the greatest worldwide prevalence [5]. However, regional differences in genotype distribution have been observed. In Japan, China and Taiwan, genotypes 1b, 2a and 2b constitute the vast majority of variants found [5,6], genotype 1a

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Accepted 7 June 1997

being very infrequent. In contrast, in southeast Asia genotypes 1a and 3 are frequently found [7]. Although the information available on genotype distribution in Africa is limited, it seems that genotype 4 predominates in wide zones of this continent [6,8,9]. However, South Africa constitutes an exception, as genotype 5 is the most frequent variety there [6,10,11]. In Occidental countries (Europe, the USA and Australia) and in South America genotypes 1, 2 and 3 are the most prevalent [5,6,8,12–14].

Another aspect studied has been the relation of prevalences to risk factors, with differences being revealed by the studies performed. Okamoto et al [7] found different prevalences of genotypes 1b and 2a in blood donors and Japanese patients with non-A, non-B hepatitis. Driesel et al [15] described an elevated prevalence of type 3a in IVDU patients.

The objective of our study was to ascertain the prevalence of different HCV genotypes in our health area, as well as the possible influence of risk factors on their distribution and their relation to hepatic disease.

PATIENTS AND METHODS

In this study, 216 patients diagnosed as having hepatitis C were included. All of them presented antibodies against HCV (anti-HCV) and HCV RNA detectable in sera by reverse transcription–polymerase chain reaction (RT–PCR). Liver biopsy was performed in 37 patients.

The distribution of patients by risk factors was as follows: 63 (29.1%) were intravenous drug users (IVDUs) (30.5±4.6 years), 44 (20.3%) had received transfusions (48.3±14.9 years) and 30 (13.8%) were hemodialyzed (52.4±15.5 years), and in 79 cases (36.5%) no known risk factor was found (55.6±15.4 years). All patients underwent testing for the presence of HBs antigen and IgM against hepatitis A virus (IMx, Abbott Laboratories, Chicago, IL, USA); the results were negative in both cases for all subjects.

A second-generation enzyme immunoassay (EIA) (Abbott Laboratories, Chicago, IL, USA) was used to screen for antibodies against HCV. Immunoblot (Deciscan HCV, Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) was used to confirm antibodies against HCV. This test uses recombinant proteins derived from the core (C1) and NS3 regions, and synthetic peptides derived from the core (C2) and NS4 regions. To detect HCV RNA, an RT–PCR (Amplicor HCV, Roche Diagnostic Systems, Inc., Branchburg, NJ, USA) was used. A reverse hybridization test of the amplification obtained by RT–PCR (INNO-LiPA HCV, Innogenetics, Belgium) was used for HCV genotyping. Specific oligonucleotide probes derived from the non-coding region 5′ are immobilized on nitrocellulose strips by a poly-T glue. The amplifications, previously marked with biotin, are incubated with the strips at 50°C to permit their hybridization with the bound probes. After washing, they are incubated with streptavidin conjugated with alkaline phosphatase at room temperature. Color development is performed by addition of nitroblue tetrazolium (NBT) and bromochloroindol phosphate (BCIP). Each strip has a total of 15 bands. The first is used as a control of the addition of conjugate. The second contains a universal probe which hybridizes with the amplifications of any genotype. The following 13 bands allow differentiation of the following HCV genotypes and subtypes according to the scheme of Simmonds et al [16]: 1, 1a, 1b, 2, 2a/2c, 2b, 3a, 3b, 4a and 5a. All tests were performed according to the manufacturers' instructions.

Statistical analysis of the results was performed by the SAS program for personal computers (SAS Institute Inc., Cary, NC, USA). Fisher's exact test (two-tail) was used for comparison of proportions in the standard two-way contingency tables. Non-parametric tests (ANOVA-based ranks) were used in comparison of measurements. Values of $p<0.05$ were considered statistically significant.

RESULTS

The dominant genotype was type 1, found in 164 of the patients studied (75.9%); the next most frequent was 3a, present in 24 patients (11.1%) (Table 1). Genotypes 4a and 2 were seldom found, being present in only eight (3.7%) and two (0.9%) patients respectively. In nine cases (4.1%), the genotype was impossible to determine with the test used. Within genotype 1 the greatest prevalence corresponded to subtype 1b, present in 104 patients (48.1%), while subtype 1a was found in 46 patients (21.3%). In 14 cases (6.4%) classified as genotype 1 it was impossible to determine the subtype. Likewise, in one of the two patients in whom genotype 2 was found, the subtype could not be determined, and the remaining case belonged to subtype 2a/2c. A mixed infection was found in nine patients (4.1%): in one case with genotypes 1 and 2, four cases with subtypes 1a and 1b, and four cases with genotypes 1a and 4a.

Table 1 shows genotype distribution according to risk factors. Genotype 1 was dominant in all groups, with percentages varying from 60.3% in the IVDU patients to 100% in the hemodialyzed patients. Analysis of the distribution of subtypes 1a and 1b by risk factor showed that the subtype predominant in IVDU patients was 1a (42.8%). However, subtype 1b was the most prevalent in patients of unknown risk (62.0%) and in

Table 1 Genotype distribution by risk factors

Genotype	IVDU No. (%)	Transfused No. (%)	Hemodialysis No. (%)	Unknown No. (%)	Total No. (%)
1	38 (60.3)	37 (84.0)	30 (100)	59 (74.6)	164 (75.9)
1-NS	1 (1.5)	3 (6.8)	3 (10.0)	7 (8.8)	14 (6.4)
1a	27 (42.8)	4 (9.0)	12 (40.0)	3 (3.8)	46 (21.3)
1b	10 (15.8)	30 (68.1)	15 (50.0)	49 (62.0)	104 (48.1)
2	1 (1.5)	1 (2.2)			2 (0.9)
2-NS		1 (2.2)			1 (0.4)
2a/2c	1 (1.5)				1 (0.4)
3a	13 (20.6)	1 (2.2)		10 (12.6)	24 (11.1)
4a	5 (7.9)	1 (2.2)		2 (2.5)	8 (3.7)
NG	3 (4.7)	2 (4.5)		4 (5.0)	9 (4.1)
Mixed	3 (4.7)	2 (4.5)		4 (5.0)	9 (4.1)
1a+1b		2 (4.5)		2 (2.5)	4 (1.8)
1a+4a	3 (4.7)			1 (1.2)	4 (1.8)
1+2				1 (1.2)	1 (0.4)
Total	63 (100)	44 (100)	30 (100)	79 (100)	216 (100)

1-NS, 2-NS=genotypes 1 or 2 non-subtypeable with the test used.
NG=non-genotypeable with the test used.

patients with blood-transfusion antecedents (68.1%). In the 30 hemodialyzed patients the prevalence of subtype 1a was 40.0% and that of subtype 1b was 50.0%. On comparing the prevalence of subtypes 1a and 1b in the different risk groups studied, we found that the prevalence of subtype 1a was significantly higher in IVDU patients (42.8%) than in patients with transfusion antecedents (9.0%) ($p<0.001$) or in those with unknown risk factors (3.8%) ($p<0.001$). In these last two groups, subtype 1b predominated. In the rest of the comparisons, no statistically significant differences ($p>0.05$) were found. Likewise, when the prevalence of genotype 3a in IVDU patients (20.6%) was compared with that in unknown risk factor patients (12.6%), no statistically significant differences ($p=0.253$) were found. However, comparing type 3a prevalence in IVDU patients (20.6%) with that in non-IVDU patients (7.6%) did yield statistically significant differences ($p=0.016$).

On the other hand, patients with genotype 3a presented the lowest average age (32.7±8.1 years). In addition, genotype 3a was associated with lower average age than that of patients with genotype 1 non-subtypeable (54.7±14.9 years), genotype 1b (53.1 ±15.8 years) and mixed genotype 1a+1b (61.2±11.3 years) ($p<0.05$ in all cases) (Table 2). Subtype 1a was detected in a younger age group (37.9±15.5 years) than was subtype 1b ($p<0.05$). In addition, the relation between genotypes and age in IVDU and non-IVDU patients was analyzed. Non-IVDU patients having genotype 3a showed lower average age (36.4±9.4 years) than non-IVDU patients infected with other genotypes. Furthermore, type 3a was associated with a lower average age than infection with genotype 1b (55.5±14.6 years) ($p<0.05$). However, no statistically significant differences were found between the average ages of the IVDU patients with the different genotypes (Table 2).

On the other hand, we analyzed the relationship between genotypes and ALT levels in patients with

Table 2 Average age of patients grouped by genotype

Genotypes	IVDU Mean±SD (no.)	Non-IVDU Mean±SD (no.)	Total Mean±SD (no.)
1-NS	28 (1)	56.8±13.3 (13)	54.7±14.9 (14)
1a	30.6±4.6 (27)	48.2±19.5 (19)	37.9±15.5 (46)
1b	30.4±4.1 (10)	55.5±14.6 (94)	53.1±15.8 (104)
2-NS	–	32 (1)	32 (1)
2a/2c	30 (1)	–	30 (1)
3a	29.6±5.6 (13)	36.4±9.4* (11)	32.7±8.1** (24)
4a	34.2±2.3 (5)	53.6±11.1 (3)	41.5±11.8 (8)
NG	27.6±5.5 (3)	49.5±14.1 (6)	42.2±15.8 (9)
1a+1b	–	61.2±11.3 (4)	61.2±11.3 (4)
1a+4a	31.6±5.5 (3)	42 (1)	34.2±6.8 (4)
1+2	–	47 (1)	47 (1)
Total	30.5±4.6 (63)	52.9±15.5 (153)	46.4±16.7 (216)

1-NS, 2-NS=genotypes 1 or 2 non-subtypeable with the test used.
NG=non-genotypeable with the test used.
*$p < 0.05$ type 3a versus type 1b. **$p < 0.05$ type 3a versus 1-NS, versus 1b, versus 1a+1b.

Table 3 Patients with pathologic ALT levels and average ALT levels by genotype

Genotypes[a]	No.	ALT[b] Mean±SD
1-NS	12	166.9±152.3
1a	33	116.2±66.5
1b	85	125.1±85.5
2a/2c	1	48
3a	23	148.4±81.9
4a	5	203.4±208.0
NG	7	98.0±35.1
1a+1b	4	155.0±77.2
1a+4a	3	149.3±97.7

[a]1-NS=genotype 1 non-subtypeable with the test used.
NG=non-genotypeable with the test used.
[b]Average ALT levels in patients with elevated ALT ($p < 0.05$ in all cases).

Table 4 Distribution of hepatic biopsy patterns by genotype

Genotype	CH No.	MC No.	PCH No.	ACH No.	Cirrhosis No.	Carcinoma No.	GH No.
1-NS				3			
1a	1		2	1			1
1b		2	1	9	6	1	
2-NS			1				
3a		1	2	1			
4a				1			
1a+1b				1	1		
1a+4a				2			
Total	1	3	6	18	7	1	1

CH=chronic hepatitis of non-specified histologic degree; MC=minimal changes; PCH=persistent chronic hepatitis; ACH=active chronic hepatitis; GH=granulomatous hepatitis; 1-NS, 2-NS=non-subtypeable with the test used.

pathologic levels of ALT. No significant differences were found ($p>0.05$ in all cases) when the ALT levels were compared in the same genotype groups (Table 3).

Liver biopsy was performed in 37 patients. In 18 (48.6%) of these, a pattern of active chronic hepatitis was found, in seven (18.9%) hepatic cirrhosis, in six (16.2%) persistent chronic hepatitis, and in another three only minimal changes (8.1%). Finally, one case each of hepatocarcinoma, of granulomatous chronic hepatitis (probably due to toxicity through isoniazid) and of non-classified chronic hepatitis was found. Table 4 presents the distribution of histologic patterns according to genotype. Genotype 1b was found in six of the seven cases of cirrhosis (85.7%) and in nine of the 18 cases (50.0%) of active chronic hepatitis.

DISCUSSION

Our results show that in the Spanish population the highest prevalence corresponded to genotype 1b, with 48.1% of the cases, followed by genotype 1a, with 22.3%, and genotype 3a, with 11.1%. These data show the greater prevalence of genotype 1b in Spain, just as in other Occidental countries [5,6,17,18], apart from the USA [5] and the north of Europe [6,19] where genotype 1a is the most frequently described. In contrast to our results, Bravo et al [19] found genotype 1a to be the most prevalent, although this difference could be due to the small number of non-IVDU patients in their study (18 cases). On the other hand, although in our study genotype 3a was the third most frequent (following types 1b and 1a), its prevalence was lower than that found in other European countries such as Scotland [12], Sweden [19] or France [18], where genotype 3a prevalence is over 20%.

Genotype 2 is present only rarely in our health area. The presence of genotype 4a among Spanish people (eight cases, 3.7%) is worth noting, as it is rarely described in countries of western Europe [6, 8, 12, 18].

The presence of infection by more than one genotype was infrequent in our study (nine cases, 4.1%), in contrast to the results of Bravo et al [20], who found 43.8% multiple infections. Mixed infections are rarely mentioned in the literature, and the majority of authors report multiple infection percentages similar to ours [17,18,21,22]. However, the significance of multiple infections is debatable: on the one hand, a large number of mixed patterns are due to cross-reactions, while on the other hand, current genotyping techniques do not detect multiple infections reliably and tend to identify principally the dominant genotypes [23].

On the other hand, an association of some genotypes with specific risk groups seems to exist. In Japan Okamoto et al [7] found genotype 1b (III of Okamoto) to be more prevalent in patients with non-A, non-B hepatitis. These authors also found genotype 1a to be more frequent in hemophiliacs, attributing this to the US origin of the coagulation factors received. In a group of German patients, Driesel et al [15] found genotype 1b to be the most frequent among all the patients, with an elevated prevalence of genotype 3a in the IVDU group. In our study we have found a clear distribution of genotypes according to risk factors: among patients of unknown risk and those with blood-transfusion antecedents, genotype 1b was the most prevalent (62.0% and 68.1%, respectively); among hemodialyzed patients no statistical differences were found between genotypes 1a and 1b (40.0% and 50.0% respectively), while among IVDU patients genotypes 1a (42.8% of all cases) and 3a (21.6%) were the most prevalent.

The low prevalence of genotype 3a in IVDU patients found both in our study and in that of Bravo et al [20] (11.5%) stands out in contrast to the prevalence observed in other nearby European countries such as France (63%) [18] and Italy (64.3%) [21]. Similar differences are found if we compare the prevalences of genotype 3a among the non-IVDU patients of these countries with our results. We feel that the low prevalence of type 3a compared with the levels existing in other European countries may be due to the later introduction of this genotype into Spain. This could be supported by the fact that patients having genotype 3a presented the lowest average age (32.7±8.1 years), whether the totality of patients studied is considered or the non-IVDU (36.4±9.4 years) and IVDU (30.6± 5.6) patient groups are analyzed separately.

The discovery of the genetic diversity of HCV gave rise to the hypothesis that the greater or lesser severity of the clinical picture and its evolution were related to specific genotypes [24]. The studies performed to date are not conclusive. McOmish et al [13] found a greater frequency of abnormalities of hepatic function in patients infected with type 3 than in those infected with types 1 and 2, although they doubt the true pathogenic significance of this fact. Dusheiko et al [8] suggest the association of genotype 1 with more serious histologic lesions in the liver. In contrast, other authors [25] suggest that a relation between genotypes and severity of hepatic disease is not very likely. On the other hand, although the frequency with which distinct degrees of histologic lesions are found varies among the different studies published [17,26–28], in conjunction persistent chronic hepatitis (PCH) and active chronic hepatitis (ACH) are the most frequent lesions, followed by cirrhosis [29]. In our study, ACH was the most frequent (18 of 37 cases), followed by cirrhosis (seven cases), but PCH was found in only five cases. Genotype 1b infection has also been frequently associated with the most severe histologic lesions of the liver (especially cirrhosis and hepatocellular carcinoma) [22,24,30,31]. We have found similar results, as the six cirrhosis patients were infected by genotype 1b. However, given that genotype 1b is predominant in our health area, it is to be expected that this genotype would appear with greater frequency in this type of histologic lesion. In any case, it is important to point out that all the patients with hepatic cirrhosis presented genotype 1b.

Acknowledgments

We wish to thank Dr Francisco Martín Rodríguez for his assistance in the elaboration and interpretation of the statistical study, and C. J. Nolan for her help in translating.

References

1. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244: 359–62.
2. Kubo Y, Takeuchi K, Boonmar S, et al. A cDNA fragment of hepatitis C virus isolated from an implicated donor of post-transfusion non-A, non-B hepatitis in Japan. Nucleic Acids Res 1989; 17: 10367–72.
3. Takeuchi K, Boonmar S, Kubo Y, et al. Hepatitis C viral cDNA clones isolated from a healthy carrier donor implicated in posttransfusion non-A, non-B hepatitis. Gene 1990; 91: 287–91.
4. Takeuchi K, Kubo Y, Boonmar S, et al. Nucleotide sequence of core and envelope genes of the hepatitis C virus genome derived directly from human healthy carriers. Nucleic Acids Res 1990; 18: 4626.
5. Takada N, Takase S, Takada A, Takayasu D. Differences in the hepatitis C virus genotypes in different countries. J Hepatol 1993; 17: 277–83.
6. Davidson F, Simmonds P, Fergurson JC, et al. Survey of major genotypes and subtypes of hepatitis C virus using RFLP of sequences amplified from the 5′ non-coding region. J Gen Virol 1995; 76: 1197–204.
7. Okamoto H, Sugiyama Y, Okada S, et al. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. J Gen Virol 1992; 73: 673–9.
8. Dusheiko G, Schmilovitz-Weiss H, Brown D, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. Hepatology 1994; 19: 13–18.
9. Bukh J, Purcell RH, Miller RH. At least 12 genotypes of hepatitis C virus predicted by sequence analysis of the putative E1 gene of isolates collected worldwide. Proc Natl Acad Sci USA 1993; 90: 8234–8.
10. Simmonds P, Holmes EC, Cha TA, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol 1993; 74: 2391–9.
11. Cha TA, Beall E, Irvina B, et al. At least five related, but distinct, hepatitis C viral genotypes exist. Proc Natl Acad Sci USA 1992; 89: 7144–8.
12. McOmish F, Yap PL, Dow BC, Follet EAC, Saleed AA, Simmonds P. Geographical distribution of hepatitis C virus genotypes in blood donors—an international collaborative survey. J Clin Microbiol 1994; 32: 884–92.
13. McOmish F, Chan S-W, Dow BC, et al. Detection of three types of hepatitis C virus in blood donors: investigation of type-specific differences in serological reactivity and rate of alanine aminotransferase abnormalities. Transfusion 1993; 33: 7–13.
14. Pontisso P, Ruvoletto MG, Nicoletti M, et al. Distribution of three major hepatitis C virus genotypes in Italy. A multicentre study of 495 patients with chronic hepatitis C. J Viral Hepatitis 1995; 2: 33–8.
15. Driesel G, Wirth D, Stork K, Baungorten R, Sucker U, Schreier E. Hepatitis C virus (NCV) genotype distribution

in German isolates: studies on the sequence variability in the E2 and NS5 region. Arch Virol 1994; 139: 379–88.
16. Simmonds P, Alberti A, Alter HJ, et al. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology 1994; 19: 1321–4.
17. Nousbaum JB, Pol S, Nalpas B, Laudais P, Berthelot P, Brechot C. Hepatitis C virus type 1b (II) infection in France and Italy. Collaborative study group. Ann Intern Med 1995; 122: 161–8.
18. Pawlotsky JM, Tsakiris L, Roudot-Thoraval F, et al. Relationship between hepatitis C virus genotyping and sources of infection in patients with chronic hepatitis C. J Infect Dis 1995; 171: 1607–10.
19. Bravo R, Soriano V, Garcia-Samaniego J, et al. Hepatitis C virus genotypes in different risk populations in Spain. J Infect Dis 1996; 183: 509–10.
20. Shev S, Widell A, Foberg U, et al. HCV genotypes in Swedish blood donors as correlated to epidemiology, liver disease and hepatitis C virus antibody profile. Infection 1995; 23: 253–7.
21. Ravaggi A, Rossini A, Mazza C, Puoti M, Marin MG, Cariani E. Hepatitis C virus genotypes in Northern Italy: clinical and virological features. J Clin Microbiol 1996; 34: 2922–5.
22. Silini E, Bono F, Cividini A, et al. Differential distribution of hepatitis C virus genotypes in patients with and without liver function abnormalities. Hepatology 1995; 21: 285–90.
23. Lau JYN, Mizokami M, Kolberg JA, et al. Application of six hepatitis C virus genotyping systems to sera from chronic hepatitis C patients in the United States. J Infect Dis 1995; 171: 281–9.
24. Simmonds P. Variability of hepatitis C virus genome. In Reesink HW, ed. Hepatitis C virus. Basel: Karger, 1994: 12–35.
25. Yamada M, Kakumu S, Yoshioka K, et al. Hepatitis C virus genotypes are not responsible for development of serious liver disease. Dig Dis Sci 1994; 39: 234–9.
26. Alter HJ. Chronic consequences of non-A, non-B hepatitis. In Seef LB, Lewis JH, eds. Current perspectives in hepatology. New York: Plenum Publishing Corporation, 1989: 83–97.
27. Esteban JI, López-Talavera JC, Genesca J, et al. High rate of infectivity and liver disease in blood donors with antibodies to hepatitis C virus. Ann Intern Med 1991; 115: 443–9.
28. Genesca J, Esteban JL, Alter HJ. Blood-borne nonA, nonB hepatitis: hepatitis C. Semin Liver Dis 1991; 11: 147–64.
29. Cordoba J, Camps J, Esteban JI. The clinical picture of acute and chronic hepatitis C. In Reesink HW, ed. Hepatitis C virus. Basel: Karger, 1994: 69–88.
30. Pozzato G, Kaneko S, Moreti M, et al. Different severity of chronic liver disease. J Med Virol 1994; 43: 291–6.
31. Feray C, Gigou M, Samuel D et al. Influence of the genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. Gastroenterology 1995; 108: 1088–96.